

We Claim:

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- ~~1.~~ A method for assessing muscle damage in a subject, comprising:
obtaining a biological sample from a subject being assessed for muscle damage;
and
evaluating for the presence or absence of one or more different myofilament protein modification products in the biological sample, at least one of said myofilament protein modification products being a chemical adduct of a myofilament protein; and
wherein the presence of at least one myofilament protein modification product in the biological sample is indicative of muscle damage in said subject.
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- ~~2.~~ The method of claim 1, further comprising the step of assessing the amount of the one or more different myofilament protein modification products present in the biological sample, as an indication of the extent of muscle damage in the subject.
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- ~~3.~~ The method of claim 1, wherein the evaluating step comprises detecting the presence of at least two different myofilament protein modification products in the biological sample.
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- ~~4.~~ The method of claim 3, further comprising the step of assessing the amounts of said at least two different myofilament protein modification products present in the biological sample, and comparing the amounts as an indication of the extent of muscle damage in the subject.
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- ~~5.~~ The method of claim 3, wherein said at least two different myofilament protein modification products are from the same protein.
- ~~6.~~ The method of claim 3, wherein said at least two different myofilament protein modification products are from different proteins.

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8. The method of claim 6, further comprising the step of assessing the ratio of said at least two different myofilament protein modification products, as an indication of the extent of muscle damage in the subject.

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9. The method of claim 1, wherein the step of evaluating for the presence or absence of a myofilament protein modification product comprises incubating the biological sample with a compound which specifically binds to the myofilament protein modification product, under conditions which allow the compound to form a complex with the myofilament protein modification product, and detecting the complex.

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10. The method of claim 8, wherein the compound is selected from the group consisting of an antibody, a functional fragment of an antibody, a protein, a protein fragment, a peptide, and a peptidomimetic.

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11. The method of claim 8, wherein the complex is detected by assaying for the presence of a label.

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12. The method of claim 8, wherein the compound is labelled with an enzyme which is detected by measuring enzymatic activity associated therewith.

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13. The method of claim 11, wherein the enzyme is selected from the group consisting of alkaline phosphatase, horseradish peroxidase, luciferase, beta-galactosidase, lysozyme, glucose-6-phosphate dehydrogenase, lactate dehydrogenase, and urease.

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14. The method of claim 8, wherein the compound is immobilized on a solid phase.

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15. The method of claim 13, wherein the solid phase is a plastic surface.

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16. The method of claim 1, wherein the muscle is selected from the group consisting of cardiac muscle and skeletal muscle.

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16. The method of claim 15, wherein the muscle damage is due to at least one condition selected from the group consisting of hypoxia, hypoxemia, ischemia, and reperfusion.

17. The method of claim 16, wherein the muscle damage is reversible.

18. The method of claim 16, wherein the muscle damage is irreversible.

19. The method of claim 1, wherein said one or more myofilament protein modification product is from at least one myofilament protein selected from the group consisting of troponin I, troponin T, troponin C, α -actinin, actin, tropomyosin, desmin, myosin light chain 1, myosin light chain 2, and myosin light chain 3.

20. The method of claim 19, wherein at least one of the myofilament protein modification products is a protein-protein complex comprising at least two polypeptides, at least one of said polypeptides being an intact protein or a fragment of a protein selected from the group consisting of troponin I, troponin T, troponin C, α -actinin, actin, tropomyosin, desmin, myosin light chain 1, myosin light chain 2, and myosin light chain 3.

21. The method of claim 1, wherein at least one of the myofilament protein modification products is a degradation product of a myofilament protein selected from the group consisting of troponin I, troponin T, troponin C, α -actinin, actin, tropomyosin, desmin, myosin light chain 1, myosin light chain 2, and myosin light chain 3.

22. The method of claim 19, wherein the chemical adduct of a myofilament protein is a myofilament protein modified by post-translational modification.

23. The method of claim 22, wherein the post-translational modification is selected from the group consisting of phosphorylation, glycosylation, myristylation, phenylation, acetylation, nitrosylation, and sulphation.

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~~24.~~ The method of claim 20, wherein the chemical adduct of a myofilament protein is a protein-protein complex modified by post-translational modification.

5 ~~25.~~ The method of claim 24, wherein the post-translational modification is selected selected from the group consisting of phosphorylation, glycosylation, myristylation, phenylation, acetylation, nitrosylation, and sulphation.

10 ~~26.~~ The method of claim 21, wherein the chemical adduct of a myofilament protein is a degradation product of a myofilament protein modified by post-translational modification.

~~27.~~ The method of claim 26, wherein the post-translational modification is selected from the group consisting of phosphorylation, glycosylation, myristylation, phenylation, acetylation, nitrosylation, and sulphation.

~~28.~~ The method of claim 8, wherein the muscle is cardiac muscle and the myofilament protein modification product is phosphorylated troponin I.

29. The method of claim 28, wherein the compound binds to a region of troponin I comprising all or a portion of the amino acid sequence from residue 194 to residue 210.

30. The method of claim 28, wherein the compound binds to a region of troponin I comprising all or a portion of the amino acid sequence from residue 1 to residue 193.

31. The method of claim 8, wherein the myofilament protein is myosin light chain 1.

32. The method of claim 31, wherein the compound binds to a region of myosin light chain 1 comprising all or a portion of the amino acid sequence from residue 20 to residue 199.

33. The method of claim 31, wherein the compound binds to a region of myosin light chain 1 comprising all or a portion of the amino acid sequence from residue 1 to residue 19.

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34. The method of claim 1, wherein the biological sample is selected from the group consisting of cardiac muscle tissue, a component of cardiac muscle tissue, blood, blood serum, blood plasma, skeletal muscle tissue, a component of skeletal muscle tissue, and urine.

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~~35.~~ A method for assessing muscle damage in a subject, comprising:
obtaining at least two biological samples from a subject being assessed for muscle damage; and
evaluating for the presence or absence of one or more myofilament protein modification products in the biological samples;
wherein said biological samples are not obtained simultaneously; and
wherein the presence of one or more myofilament protein modification products in at least one of said biological samples is indicative of muscle damage in the subject.

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~~36.~~ The method of claim 35, wherein at least one of the myofilament protein modification products is a chemical adduct of a myofilament protein.

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~~37.~~ The method of claim 35, further comprising assessing a change with time in the presence or amount of one or more myofilament protein modification products in the biological samples, as an indication of the extent of muscle damage in the subject.

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~~38.~~ The method of claim 35, wherein the evaluating step comprises detecting the presence of at least two different myofilament protein modification products in the biological samples.

~~39.~~ The method of claim 38, further comprising the step of assessing a change with time in the amounts of said at least two different myofilament protein modification products present in the biological samples, as an indication of the extent of muscle damage in the subject.

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~~40.~~ The method of claim 38, wherein said at least two different myofilament protein modification products are from the same protein.

~~41.~~ The method of claim 38, wherein said at least two different myofilament protein modification products are from different proteins.

42. ~~A~~ kit for assessing the extent of muscle damage in a biological sample obtained from a subject, comprising:
a compound which specifically binds to a chemical adduct of a myofilament protein to form a complex; and
instructions explaining how to use the kit to assess muscle damage in a biological sample obtained from a subject.

43. The kit of claim 42, wherein the compound is selected from the group consisting of an antibody, a functional fragment of an antibody, a protein, a protein fragment, a peptide, and a peptidomimetic.

44. The kit of claim 43 further comprising a label which binds to the complex.

45. The kit of claim 44 further comprising at least one reagent for detecting the label.

46. The kit of claim 42, wherein the chemical adduct of a myofilament protein is a myofilament protein modified by a process selected from the group consisting of phosphorylation, glycosylation, myristylation, phenylation, acetylation, nitrosylation, and sulphation.

47. The kit of claim 46, wherein the myofilament protein is selected from the group consisting of troponin I, troponin T, troponin C, α -actinin, actin, tropomyosin, desmin, myosin light chain 1, myosin light chain 2, and myosin light chain 3.

48. The kit of claim 46, wherein the myofilament protein is a protein-protein complex comprising at least two polypeptides, at least one of said polypeptides being an intact protein or a fragment of a protein selected from the group consisting of troponin I, troponin T, troponin C, α -actinin, actin, tropomyosin, desmin, myosin light chain 1, myosin light chain 2, and myosin light chain 3.

49. The kit of claim 44, wherein the label is an enzyme which is detected by measuring the enzymatic activity associated therewith.

50. The kit of claim 49, wherein the enzyme is selected from the group consisting of alkaline phosphatase, horseradish peroxidase, luciferase, beta-galactosidase, lysozyme, glucose-6-phosphate dehydrogenase, lactate dehydrogenase, and urease.

51. A method of screening for an agent which modulates the level of a chemical adduct of a myofilament protein present in a biological sample, comprising:
obtaining from a subject a biological sample containing a chemical adduct of a myofilament protein;
testing at least a portion of the biological sample with an agent; and
determining the effect of the agent on the level of the chemical adduct of a myofilament protein in the biological sample.

52. The method of claim 51, wherein the level of the chemical adduct of a myofilament protein is determined using a compound which binds specifically to the chemical adduct of a myofilament protein.

53. The method of claim 51, wherein the chemical adduct of a myofilament protein is a myofilament protein modified by a process selected from the group consisting of phosphorylation, glycosylation, myristylation, phenylation, acetylation, nitrosylation, and sulphation.

54. The method of claim 53, wherein the myofilament protein is selected from the group consisting of troponin I, troponin T, troponin C, α -actinin, actin, tropomyosin, desmin, myosin light chain 1, myosin light chain 2, and myosin light chain 3.

55. The method of claim 53, wherein the myofilament protein is a protein-protein complex comprising at least two polypeptides, at least one of said polypeptides being an intact protein or a fragment of a protein selected from the group consisting of troponin I, troponin T, troponin C, α -actinin, actin, tropomyosin, desmin, myosin light chain 1, myosin light chain 2, and myosin light chain 3.

56. A method for assessing muscle damage in a subject, comprising:
obtaining a biological sample from a subject being assessed for muscle damage;
incubating the biological sample with at least one compound which specifically binds to one or more different myofilament proteins or myofilament protein modification products present in the sample, under conditions which allow the compound to form one or more complexes with the myofilament proteins or myofilament protein modification products;
detecting said one or more complexes; and
characterizing the profile of said one or more myofilament proteins or myofilament protein modification products contained in said one or more complexes, as an indication of the extent or type of muscle damage in the subject;
wherein at least one of the myofilament protein modification products is a chemical adduct of a myofilament protein.

57. The method of claim 56, wherein the detecting step comprises detecting at least one complex containing two different myofilament protein modification products.

58. The method of claim 56, wherein the chemical adduct of a myofilament protein is a myofilament protein modified by a process selected from the group consisting of phosphorylation, glycosylation, myristylation, phenylation, acetylation, nitrosylation, and sulphation.

59. The method of claim 58, wherein the myofilament protein is selected from the group consisting of troponin I, troponin T, troponin C, α -actinin, actin, tropomyosin, desmin, myosin light chain 1, myosin light chain 2, and myosin light chain 3.

5 60. The method of claim 58, wherein the myofilament protein is a protein-protein complex comprising at least two polypeptides, at least one of said polypeptides being an intact protein or a fragment of a protein selected from the group consisting of troponin I, troponin T, troponin C, α -actinin, actin, tropomyosin, desmin, myosin light chain 1, myosin light chain 2, and myosin light chain 3.

10 61. The method of claim 56, wherein said one or more complexes is detected in an ELISA.

15 62. The method of claim 56, wherein the step of characterizing the profile of said one or more different myofilament proteins or myofilament protein modification products comprises performing an immunoblot analysis.

20 63. The method of claim 56, wherein the step of characterizing the profile of said one or more different myofilament proteins or myofilament protein modification products comprises performing an HPLC analysis.

25 64. The method of claim 56, wherein the step of characterizing the profile of said one or more different myofilament proteins or myofilament protein modification products comprises performing a polyacrylamide gel electrophoresis analysis.

65. The method of claim 56, wherein the step of characterizing the profile of said one or more different myofilament proteins or myofilament protein modification products comprises comparing the sizes of the proteins or modification products.

